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BROOD SEX RATIO VARIATION IN MOUNTAIN BLUEBIRDS (*SIALIA CURRUCOIDES*): EFFECTS OF ATTRACTIVENESS AND ENVIRONMENT

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CURRUCOIDES*): EFFECTS OF ATTRACTIVENESS AND ENVIRONMENT**

by

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ABSTRACT

Sex allocation theory predicts that females should bias the sex ratio of their offspring in response to differences in the reproductive value of sons and daughters. Offspring reproductive value may vary as a result of mate attractiveness, mate condition, or habitat quality; therefore, females should bias sex ratio in response to these attributes. Male plumage colouration, for example, may signal the direct (e.g., parental care) and indirect (e.g., good genes) benefits a female can gain by mating with a particular male. If males inherit good genes and receive greater parental care from a high quality dad, sons will have a higher chance of reproductive success. Hence, a female mated to a high quality male may produce a male-biased brood. Male mountain bluebirds (*Sialia currucoides*) display full body UV-blue structural plumage colouration, which is associated with male attractiveness and condition. In this study, I investigated whether female mountain bluebirds produce sex-biased broods in response to mate attractiveness or environmental conditions. Brood sex ratios were related to male plumage colouration, with male-biased broods resulting when females mated with brightly coloured males. This relationship was seen only in broods produced by older females, suggesting that a female's ability to assess male plumage and bias offspring sex in response may be related to her breeding experience. Brood sex ratios also became male-biased with increasing elevation, and male-biased broods were provisioned more frequently than female-biased broods when the parents were older birds. In conclusion, these results support the hypotheses that female mountain bluebirds bias offspring sex in response to both mate attractiveness and environmental conditions.

Thesis Supervisor: Assistant Professor, Dr. Matthew Reudink

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INTRODUCTION

Sexual reproduction is common to all vertebrate life, yet the factors controlling offspring sex are variable across taxa. Sex determination in vertebrates can be classified into two general categories: environmental sex determination (ESD) and genetic sex determination (GSD) (Pike and Petrie 2003; Bachtrog et al. 2014). Under ESD, a zygote's sex is determined post-fertilization by environmental factors, such as temperature-dependent sex determination, in which gonadal differentiation is determined by ambient temperature during the egg incubation period (Janzen and Paukstis 1991; Trukhina et al. 2013). ESD is uncommon in vertebrates, and such systems are only found in some reptile and bony fish species (Trukhina et al. 2013; Bachtrog et al. 2014). GSD systems, however, are more prevalent and found in all mammals, birds and amphibians (Trukhina et al. 2013).

Through GSD, sex is determined by genetic elements, which are typically sex chromosomes (Trukhina et al. 2013; Bachtrog et al. 2014). The two main forms of GSD are the XY sex determination system, and the ZW sex determination system. In the XY sex determination system, males are the heterogametic sex, with one X sex chromosome and one Y sex chromosome, while females are the homogametic sex, with two X sex chromosomes. The XY system is found in mammals, including humans, and some amphibian, reptile and bony fish species (Trukhina et al. 2013; Bachtrog et al. 2014). Unlike XY sex determination, ZW sex determination is characterized by female heterogamy, with females defined by their one Z sex chromosome and one W sex chromosome, and males by their two Z sex chromosomes (Griffiths et al. 1998). The ZW sex determination system is found primarily in birds, but also appears in reptiles, amphibians and bony fish (Griffiths et al. 1998; Bachtrog et al. 2014).

Regardless of the sex determination system, anisogamy, or male and female gametes of different sizes, in sexually reproducing organisms results in differences in the cost of reproduction between the sexes, with eggs being more costly to produce than sperm. Female reproductive success is limited by egg production, whereas male reproductive success is limited by the number of eggs they can fertilize. As a result, males tend to experience greater reproductive variance than females. The difference in reproductive variance between the sexes has been hypothesized to influence both maternal and paternal investment in offspring (Burley 1986, 1988). Because the number of offspring a female can produce in her lifetime is limited, females should invest time and energy to maximize offspring survival and reproduction.

When the reproductive value of sons and daughters differ, a female may allocate more resources to the higher value gender to incur fitness benefits, and by doing so, bias the sex ratio of her resulting brood (Trivers and Willard 1973). Which gender is of greater reproductive value is dependent on both intrinsic factors (e.g., her own condition) and extrinsic factors (e.g., mate quality and resource availability) (Trivers and Willard 1973; Burley 1981). Because males display greater variance in reproductive success than females, a high-quality son has the potential to produce more offspring and have higher reproductive success than a daughter of the same quality. The opposite is true for low quality sons and daughters, as females almost always mate regardless of quality, while a low quality male may be unable to reproduce at all (Trivers and Willard 1973). Therefore, a female capable of producing high quality offspring would benefit more from producing sons than daughters, while a female only capable of producing low quality offspring would benefit more from producing daughters than sons (Trivers and Willard 1973). In nature, biased sex allocation in

response to parental condition and habitat quality has been documented in many vertebrates (Hardy 1997), including numerous bird species (Sheldon 1998)

In species with GSD, brood sex ratios may be adjusted either internally, in the form of biased offspring production, or externally, in the form of biased offspring mortality (Pike and Petrie 2003). Sex chromosomes segregate during meiosis in a Mendelian fashion, which should result in an equal probability of producing a son or a daughter (Pike and Petrie 2003; Bachtrog et al. 2014). Therefore, it would seem unlikely that individuals could adaptively deviate brood sex ratios from random internally (Pike and Petrie 2003). However, offspring sex ratio bias has been noted in many bird species at laying (Sheldon 1998), indicating that the ratio is adjusted internally pre- or post-ovulation (Pike and Petrie 2003).

In birds, offspring will always inherit a Z chromosome from the father, so it is the chromosome inherited from the mother that will determine offspring sex. Therefore, female birds may be able to modify the sex ratio of their resulting broods through sex specific egg development (Pike and Petrie 2003). Though the physiological mechanisms allowing females to control sex specific egg development are poorly understood, the most plausible theories suggest female hormonal control (Pike and Petrie 2003; Navara 2013).

Follicle development, ovulation and egg development in the ovaries of female birds are under the control of hormones, including follicular stimulating hormone, luteinizing hormone, testosterone, progesterone, and corticosterone (Pike and Petrie 2003; Navara 2013). In species that lay multiple eggs, follicles typically develop 24 hours out of phase with one another, resulting in a follicular size hierarchy (Pike and Petrie 2003). If the development of follicles that will ultimately give rise to males and females have differential sensitivity to hormones, different hormonal environments may affect their development and drive

preferential ovulation of follicles destined to become one sex over the other (Pike and Petrie 2003; Navara 2013).

In addition to controlling follicle development and ovulation within the ovaries, female hormones are present within the egg yolk and may affect embryo development (Pike and Petrie 2003). Yolk testosterone and estrogen levels differ between eggs containing male and female peafowl (*Pavo cristatus*), with male eggs containing more testosterone and female eggs containing more estrogen (Petrie et al. 2001). Testosterone has the potential to inhibit the development of female embryos, so yolk hormone levels may control sex-biased embryo development (Petrie et al. 2001). Because female hormone levels change in response to mate attractiveness, self condition and attractiveness, and resource availability, these factors may be reflected in ovarian and yolk hormone levels, resulting in sex specific follicle and embryo development and a biased sex ratio (Pike and Petrie 2003).

Although the physiological mechanisms underlying sex ratio bias in birds are poorly understood, the external factors associated with biased sex ratios are becoming unraveled on a species-specific basis. In species with bi-parental care, the reproductive value of male and female offspring varies as a result of differences in male and female condition/quality, parental care and territory quality (Hasselquist and Kempenaers 2002). Accordingly, females appear to bias sex ratios in response to these factors (Sheldon 1998).

Consistent with Triver and Willard's (1973) sex allocation theory, female condition has been associated with brood sex ratio in black-billed gulls (*Larus fuscus*; Nager et al. 1999), tree swallows (*Tachycineta bicolor*; Whittingham and Dunn 2000), peafowl (Pike and Petrie 2005), and yellow-legged gulls (*Larus michahellis*; Alonso-Alvarez and Velando 2003), with females in better condition producing a greater proportion of males. Though

individual quality is influenced by genetic factors, environmental factors, such as habitat conditions and resource availability, limit the maximum condition an individual can achieve. Therefore, on a high quality habitat with plentiful resources and limited intraspecific competition, females would be expected to produce more sons than daughters, while the opposite would be expected of females on low quality habitat. In accordance with these predictions, great tit (*Parus major*) broods from high quality deciduous forest habitat contained a greater proportion of male offspring than broods from low quality mixed woodland habitat (Stauss et al. 2005). In addition, western bluebirds (*Sialia mexicana*) produced more female-biased broods when local resource and interspecific competition was high (Dickinson 2004).

Much research into brood sex ratio variation in birds has focused on the response of females to variation in male attractiveness. In species with female mate choice, male attractiveness is often associated with sexually selected plumage characteristics, with attractive males being those that display more exaggerated forms of plumage (Burley 1986). Male plumage signals the direct (e.g., parental care) and indirect (e.g., good genes) benefits a female will gain by mating with a particular male (Griffith & Pryke 2006). Thus, a female mated with an attractive male should produce a male-biased brood to ensure her offspring inherit good genes and are well cared for. Because females experience relatively low variation in reproductive success, a daughter would be less impacted by the inheritance of lower quality genes than a son would be, suggesting a female-biased brood would be adaptive when a female is mated to a less attractive male. Male plumage ornamentation has been positively correlated with male-biased broods in collared flycatchers (*Ficedula*

albicollis; Ellegren et al. 1996), common yellowthroats (*Geothlypis trichas*; Taff et al. 2011), blue tits (Sheldon et al. 1999; Griffith et al. 2003), and great tits (Kölliker et al. 1999).

Although several studies have found evidence supporting avian brood sex ratio adjustment, others have failed to do so (Koenig and Dickinson 1996; Korsten et al. 2006; Delhey et al. 2007). The current literature is highly contradictory as one study will find a relationship in a certain species, while another study on the same species but a different population or during a different year will not (e.g., western bluebird: Koenig and Dickinson 1996, Dickinson 2004; blue tit: Griffith et al. 2003, Korsten et al. 2006, Delhey et al. 2007). To decipher these contradictions, multi-year studies on multiple populations need to be performed.

The purpose of this research was to investigate whether female mountain bluebirds (*Sialia currucoides*) produce sex-biased broods in response to mate attractiveness or environmental conditions. Mountain bluebirds display UV-blue plumage and are sexually dimorphic (Power and Lombardo 1996). Breeding males possess fully-body UV-blue plumage, while breeding females have more subdued brown-grey plumage, with duller blue on their rump, tail and flight feathers (Power and Lombardo 1996). Unlike carotenoid and melanin based plumage colouration that depend primarily upon pigment deposition, UV-blue plumage is structurally based and depends upon feather microstructure (Prum 2006). Consequently, an individual's nutritional state during moult affects the resulting feather structure and colour (Keyser and Hill 1999; Siefferman and Hill 2005, 2007; Doyle and Siefferman 2014). Therefore, structurally based plumage appears to be an honest signal of an individual's condition and quality (Keyser and Hill 2000; Siefferman and Hill 2003).

In a Wyoming, USA, population of mountain bluebirds, male plumage colouration was positively correlated to male wing size and total (i.e., within-pair and extra-pair) reproductive success, suggesting that brightly coloured males are in better condition and of higher quality than dull males, and that male plumage colouration is under sexual selection (Balenger et al. 2009a). Mountain bluebirds are socially monogamous and show weak negative assortative mating in regard to plumage colour (Morrison et al. 2014). Extra-pair paternity rates are high in this species (72% of broods; Balenger et al. 2009b), and males who sire extra-pair young have brighter UV-blue plumage than males who do not (Balenger et al. 2009a; O'Brien and Dawson 2011), suggesting the opportunity for sexual selection to act on male UV-blue plumage (Balenger et al. 2009b).

Because the UV-blue structural colouration of a male's plumage signals his attractiveness, condition and quality to potential mates, mountain bluebirds are well suited for investigating offspring sex ratio bias in relation to male plumage colouration. To my knowledge, brood sex ratios have not yet been studied in mountain bluebirds, although, they have been in the closely related eastern bluebird and western bluebird. Nestling sex ratios in eastern bluebirds did not deviate from unity (Lombardo 1982); however, brood sex ratios in western bluebirds became male-biased as the number of helpers-at-the-nest and local resource competition increased (Dickinson 2004). Although mountain bluebirds are not cooperative breeders, females may adjust sex ratio in response to local resource competition (i.e., habitat quality).

Mountain bluebirds are a grassland species, breeding in prairie-forest ecotones with moderate tree cover (Power and Lombardo 1996). Grassland plant communities in interior British Columbia are characterized by bunchgrass prairies at lower elevations, and increasing

deciduous and coniferous tree cover at higher elevations. Because mountain bluebirds typically breed on sites with moderate tree cover, habitat quality may increase with elevation. In a population of mountain bluebirds inhabiting the Bighorn Mountains, Wyoming, USA, both male and females provisioned their young more frequently during the late nestling period when they had nested at higher elevation sites, suggesting that prey may be more abundant at higher elevations (Johnson et al. 2006).

Mountain bluebirds are cavity nesting species and readily nest in artificial nest boxes (Power and Lombardo 1996). In our study population of mountain bluebirds, pairs nesting in hole entrance nest boxes had a greater number of nestlings and greater fledging success than birds nesting in slot entrance boxes (Bailey, unpublished data). No difference in clutch size was detected between hole and slot entrance nest boxes, indicating that the difference in fledging success is due to hole entrance boxes providing a more suitable environment for nestling growth (Bailey, unpublished data). In addition, slot entrance nest boxes appear to be actively avoided, as they are occupied at a lower frequency than hole entrance nest boxes (Bailey, unpublished data). Therefore, females occupying slot entrance boxes may recognize they are nesting in low quality sites and bias their offspring sex ratio accordingly.

Through this study, I tested the hypotheses that female mountain bluebirds adjust offspring sex ratio in response to mate attractiveness and habitat quality. Based on Burley's (1981) findings, I predicted that male-biased broods would result when female mountain bluebirds mated to males with bright UV-blue plumage. I predicted there would be relationships between habitat quality (e.g. elevation, nest box entrance type) and brood sex ratios; however, no *a priori* assumptions were made about the directionality of these relationships. Parental provisioning rates may be indicative of individual, mate, and territory

quality. Because male-biased brood should result when a female mates with a high quality male, and high quality males should provision more frequently, I predicted that both males and females would provision male-biased broods more.

MATERIALS AND METHODS

Field Methods

Field work for this project was conducted during the 2011 and 2012 breeding seasons (May-July) in the Knutsford area of Kamloops, British Columbia, Canada (885-1116 m ASL; 50°37'N, 120°19'W), using nest box routes established and maintained by the Kamloops Naturalist Club. Nest boxes were monitored every one to three days to determine first egg date, clutch size, hatch date, number of nestlings and fledging success. Five to ten days after eggs hatched, adult males and females were captured at the nest using nest box traps. We classified adults as either second-year (SY) or after-second-year (ASY) by examining the moult limits of the primary and greater coverts, as described by Pyle (1997). To evaluate individual body size and condition, we measured mass, unflattened wing chord, tail length and tarsus length. Adults were banded with a single Canadian Wildlife Service aluminum band and a unique combination of three plastic colour bands. We collected blood samples from adults and nestlings by piercing the ulnar vein and drawing 15-25 µl of blood into a micro-capillary tube. We sampled blood from nestlings 9 to 13 days after hatching and from adults at the time of banding. At the time of collection, nestlings were banded with a single Canadian Wildlife Service aluminum band.

Parental Care

Parental care was quantified by video recording provisioning trips for two-hour periods during the early (three to five days after hatching) and late (14-16 days after hatching) nestling phases, for a total of four hours per nest. Nest watch videos were recoded using a Handycam DCR-SX45 (Sony, Tokyo, Japan) or a HD Hero2 or 3 (GoPro, San Mateo, CA). All nest watch start times occurred between 06:20 and 11:15 (mean \pm SD, 08:48 \pm 92 min). Video cameras were placed on the ground approximately two metres in front of the nest box and aimed at the box entrance. The videos were analyzed by a human observer to determine provisioning rates for both the attending male and female. Provisioning rates were measured as the number of trips to the nest per hour per nestling. Female visits to the nest during the early nestling phase lasting longer than 30 s were recorded as brooding. Adults were not captured at nests within the 48 hours prior to nest watches to avoid modification of parental behaviour.

Feather Colour Analysis

At the time of capture, we collected ten rump feathers and a single R3 tail feather from each adult. Following collection, we mounted rump and tail feathers side-by-side on low-reflectance black paper. We mounted the ten rump feathers in an overlapping pattern that mimicked the way the feathers lay naturally on the bird. We stored mounted feathers in separate envelopes until spectrometer colour analysis was performed. We quantified male and female plumage colouration by measuring reflectance across the avian visual spectrum (300-700 nm) using an Ocean Optics JAZ spectrometer (Dunedin, FL) with a PX-2 xenon light source. The fiber optic probe was held in a non-reflective probe holder to measure

feathers from a standard 90° angle and 5.9 mm distance. We took ten readings for each plumage region (tail and rump) from haphazard locations within the blue regions.

Using the R-based colour analysis program RCLR v.28 (Montgomerie 2008), we calculated three colour variables for each feather reading (brightness, hue and chroma) and averaged the value for each variable over the ten readings from each feather sample. Brightness was measured as the average percent reflectance across the avian visual spectrum (300-700 nm). Chroma was measured as the proportion of reflectance within the blue range (400-510 nm) and ultraviolet range (300-400 nm) relative to the total light reflected across the avian visual range. Finally, hue was measured as the wavelength at maximum reflectance. Due to high colinearity among these three colour variables, we used principal component analysis to collapse the three variables into a single variable. Because the first principal component (PC1) was found to explain most of the variation for each plumage area, PC1 was used to represent overall colour variation (see appendix A).

Molecular Methods

Blood taken from adults and nestlings was stored in ethanol at 4°C until later DNA extraction. Total genomic DNA was extracted using the standard protocol (E.Z.N.A Blood DNA Mini Kit handbook) for the E.Z.N.A Blood DNA Mini Kit (Omega Bio-Tek, Norcross, GA) and stored at -20°C.

Nestling sex was determined by polymerase chain reaction (PCR) amplification of two homologous avian sex chromosome genes. P8 (5'-CTCCCAAGGATGAGRAAYTG-3') reverse and P2 (5'-TCTGCATCGCTAAATCCTTT-3') forward primers were used to amplify the chromo-helicase-DNA-binding (CHD) genes of the Z (CHD-Z) and W (CHD-W) sex chromosomes (Griffiths et al. 1998). The P8 and P2 primers have been designed to amplify a

region of the CHD genes that includes an intron. The amplified intron region of CHD-W is larger than that of CHD-Z, resulting in a larger CHD-W amplicon and allowing for product separation (Griffiths et al. 1998). An approximately 300 bp amplicon is produced from the CHD-Z gene and an approximately 375 bp amplicon is produced from the CHD-W gene. Following electrophoresis, males were identified by the presence of a single 300 bp band and females were identified by two bands, one 300 bp band and one 375 bp band (Fig. 1).

PCR amplification was carried out in a total volume of 25 μ l. The final reaction conditions were as follows: 10.35 μ l H₂O; 3.0 μ l (3.0 mM) MgCl; 5.0 μ l 5x buffer; 0.5 μ l nucleotide mix; 0.15 μ l *Taq* polymerase; 2.5 μ l each 10x P2 and P8 primers; 1 μ l genomic DNA. All reagents were supplied in the GoTaq PCR Core System II (Promega, Madison, WI) and PCR was performed in a MyCycler Thermocycler (Bio-Rad Laboratories, Hercules, CA). Genomic DNA was diluted to approximately 380 ng/ μ l (mean \pm SD, 366 \pm 120 ng/ μ l),

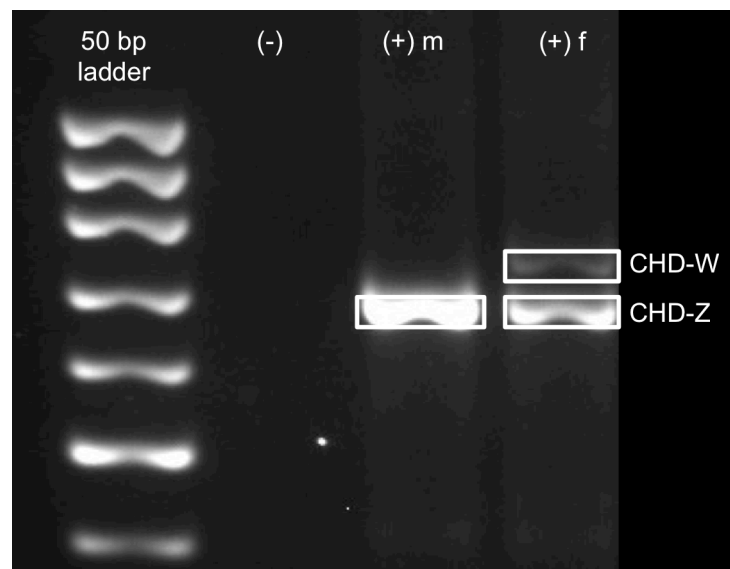


Figure 1. 2.5% agarose gel run at 90 V for 120 min. Male ((+) m) nestlings were identified by a single 350 bp band from the Z chromosome (CHD-Z), while female ((+) f) nestlings were identified by one 350 bp band from the Z chromosome and one 375 bp band from the W chromosome (CHD-W).

as this was found to be the optimal concentration for amplification (Boyda, unpublished data). An initial denaturing step at 94°C for 5 min was followed by 30 cycles of 94°C for 30 s, 51°C for 45 s, and 72°C for 45 s. A final run of 48°C for 1 min and 72°C for 5 min completed the program. PCR products were separated by gel electrophoresis for 120 min at 90 V in a 2.5% agarose gel (10 cm x 7 cm) stained with GelRed Nucleic Acid Stain (Biotium, Hayward, CA), and visualized using a Gel Doc XR system (Bio-Rad Laboratories). When tested using DNA from adult birds of known sex ($n = 32$; females, $n = 21$; males, $n = 11$), the protocol was found to have 100% accuracy (32/32 birds correctly sexed). Only DNA samples from broods of four or more offspring and from which both the male and female attending the nest had been caught and identified were included in sex ratio analysis.

Statistical Analysis

Statistical analyses were performed using JMP 12 statistical analysis software (SAS Institute, 2015). Because my data did not meet the assumptions of normality required for parametric tests, I used non-parametric correlational analysis (Spearman's rho) to determine relationships between brood sex ratios and (1) male and female plumage colouration, (2) parental provisioning rates during early and late nestling stages, (3) first egg date, and (4) habitat characteristics (elevation and nest box type). Data were analyzed both pooled and separately for male and female age classes (SY and ASY). Wilcoxon signed-rank tests were performed to determine if the population sex ratio and brood sex ratios deviated from unity, and to determine if there were differences in these ratios between years (2011/2012), age classes (SY/ASY), and nest box type (hole/slot entrance).

RESULTS

Population and Brood Sex Ratios

I determined the sex of 204 nestlings from 40 broods in the 2011 (nestlings, $n = 133$; broods, $n = 26$) and 2012 (nestlings, $n = 73$; broods, $n = 14$) breeding seasons. I could not assign gender to five nestlings from four broods because no DNA was available ($n = 3$ nestlings), or because I failed to obtain PCR products from those individuals ($n = 2$ nestlings). Overall, 52% of the offspring in the population were male, which did not differ from unity ($n = 204$, $W = 512.5$, $p = 0.49$), nor did the percentage of male offspring differ from unity within either of the two years of the study (2011: 51%, $n = 133$, $W = 100.5$, $p = 0.80$; 2012: 52%, $n = 71$, $W = 126.0$, $p = 0.41$). Brood sex ratios are expressed as the proportion of male offspring in a brood, and mean brood sex ratio did not differ from 0.50 (combined years: 0.52, $n = 40$, $W = 82.0$, $p = 0.27$; 2011: 0.51, $n = 26$, $W = 29.5$, $p = 0.46$; 2012: 0.54, $n = 14$, $W = 13.5$, $p = 0.53$) (Fig. 2). Because mean brood sex ratio did not differ between years ($n = 40$, $Z = 0.12$, $p = 0.91$), data were pooled in subsequent analyses. In addition, mean brood sex ratio did not

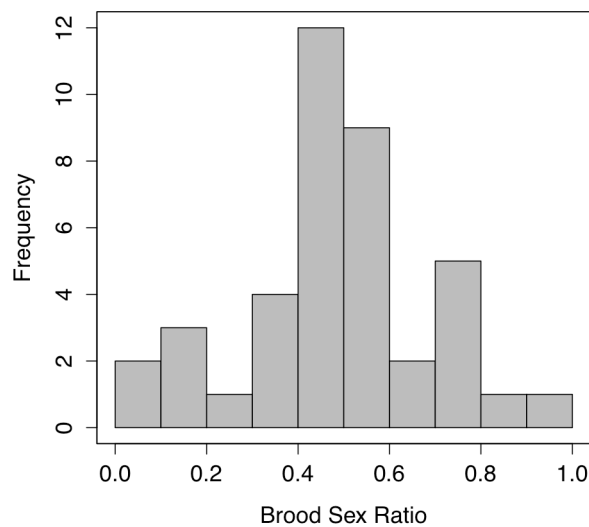


Figure 2. Distribution of brood sex ratios (proportion of male offspring) for 2011 and 2012. The mean sex ratio, 0.52, did not deviate from unity.

differ between age classes for either males (SY: 0.56, ASY: 0.50; $n = 40$, $Z = 1.48$, $p = 0.14$) or females (SY: 0.53, ASY: 0.52; $n = 40$, $Z = 0.68$, $p = 0.49$).

Parental Plumage Colour and Brood Sex Ratio

Neither male nor female rump nor tail plumage colouration were associated with brood sex ratio whether ages were pooled or unpooled for each gender respectively (see appendix B). When male plumage colouration was related to brood sex ratio with regard to female age, broods were more male-biased when ASY females mated to males with brighter tail plumage (R3 PC1) ($n = 24$, $\rho = 0.52$, $p = 0.009$) (Fig. 3); however, this relationship was not seen in SY females ($n = 15$, $\rho = -0.03$, $p = 0.92$). When male plumage colouration was related to brood sex ratio with regard to both female and male age together, pairs consisting of an ASY male and an ASY female had more male-biased broods when male tail plumage (R3 PC1) was brighter ($n = 19$, $\rho = 0.64$, $p = 0.003$) (Fig. 4), but not in any other pair age groups (ASY male/SY female: $n = 10$, $\rho = -0.42$, $p = 0.23$; SY male/ASY female: $n = 5$, $\rho = 0.30$, $p = 0.62$; SY male/SY female: $n = 5$, $\rho = 0.50$, $p = 0.39$).

Parental Provisioning Rates and Brood Sex Ratio

During the early nestling stage, older ASY males provisioned male-biased broods more frequently than female-biased broods ($n = 27$, $\rho = 0.41$, $p = 0.04$) (Fig. 5). Both male and female provisioning rates during the early nestling stage were greater in male-biased broods when both parents were ASY (male provisioning: $n = 17$, $\rho = 0.55$, $p = 0.02$; female provisioning: $n = 17$, $\rho = 0.49$, $p = 0.05$). During the late nestling stage, females mated with younger SY males provisioned female-biased broods at a higher rate than male-biased broods ($n = 8$, $\rho = -0.72$, $p = 0.04$) (Fig. 6).

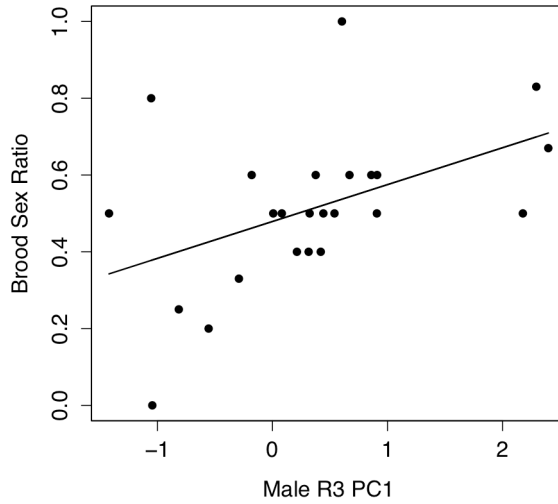


Figure 3. Broods were more male-biased when ASY females mated to males with bright UV-blue tail plumage.

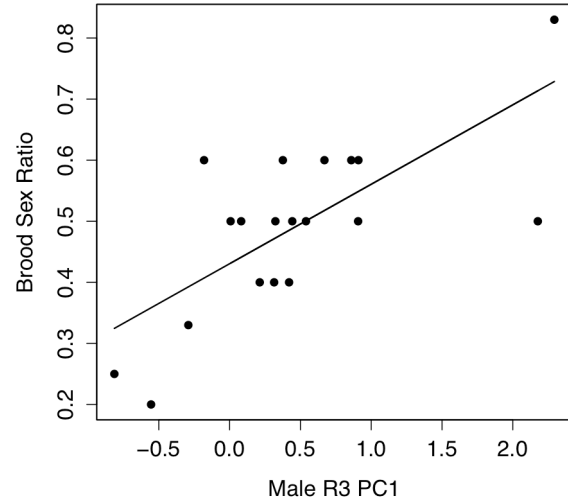


Figure 4. Pairs consisting of an ASY female and an ASY male had more male-biased broods when male UV-blue tail plumage was brighter.

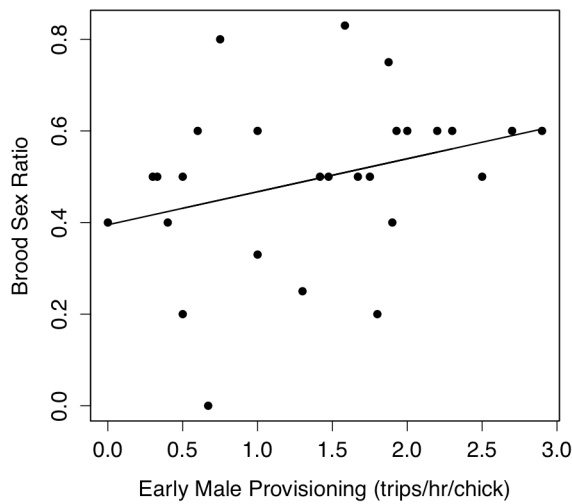


Figure 5. During the early nestling period, older ASY males provisioned male-biased broods more frequently than female-biased broods.

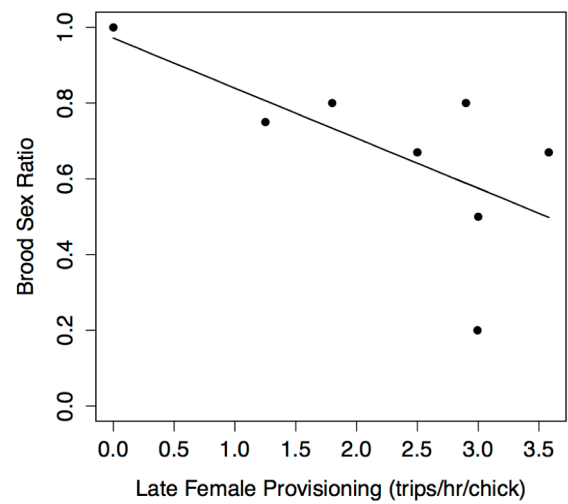


Figure 6. During the late nestling period, females mated with young SY males provisioned female-biased broods more frequently than male-biased broods.

First Egg Date and Brood Sex Ratio

I found no relationship between first egg date and brood sex ratio ($n = 40$, $\rho = 0.22$, $p = 0.17$). However, there was a significant relationship between first egg date and male rump PC1 ($n = 39$, $\rho = 0.34$, $p = 0.03$), with females mated to more brightly coloured males beginning egg laying earlier in the season.

Environment and Brood Sex Ratio

Nest box type (slot/hole entrance) was not associated with brood sex ratio ($n = 38$, $Z = 0.12$, $p = 0.90$). Elevation, however, was significantly correlated to brood sex ratio ($n = 38$, $\rho = 0.36$, $p = 0.03$), with broods from higher elevations being more male-biased.

DISCUSSION

In this study, I observed support for sex allocation in mountain bluebirds with respect to both mate quality and environmental conditions. Consistent with my predictions, male-biased broods resulted when female mountain bluebirds mated with more brightly coloured males. During the early nestling phase, male-biased broods also received more provisioning from older males, and greater male and female provisioning when both parents were older. In addition to mate quality, brood sex ratios were more male-biased at higher elevations, though there was no difference in brood sex ratio between hole and slot entrance nest boxes. Taken together, these results suggest that female mountain bluebirds may bias brood sex ratio in response to both mate attractiveness and environmental conditions.

Relationships between plumage ornamentation and brood sex ratio have been reported previously in several species. Male plumage ornamentation was positively correlated with more male-biased broods in collared flycatchers (*Ficedula albicollis*;

Ellegren et al. 1996), common yellowthroats (*Geothlypis trichas*; Taff et al. 2011), blue tits (Sheldon et al. 1999; Griffith et al. 2003), and great tits (Kölliker et al. 1999). Some studies have also found parental age to be directly related to sex allocation (Sheldon et al. 1999; Griffith et al. 2003). In blue tits, for example, older females tend to produce more male-biased broods (Griffith et al. 2003). Few studies, however, have investigated brood sex ratio in relation to both parental age and plumage colouration (Taff et al. 2011). In a population of common yellowthroats, Taff et al. (2011) found that male plumage colouration predicted offspring sex when the male was younger. Females mated to younger males with greater bib UV brightness were more likely to produce male offspring. No such relationship was found in older males or in regard to female age, however, which is contrary to the findings of my study. The UV brightness of a male common yellowthroat's bib is a reliable signal of his condition and positively influences his fertilization success, but only for young males (Freeman-Gallant et al. 2010). Taff and colleagues (2011) suggest that because these relationships are limited to young males, only females mated with young males would realize the benefits to producing sex-biased offspring.

In my study, male-biased broods resulted when older female mountain bluebirds mated to males with brighter tail UV-blue tail plumage. This relationship still held when only pairs consisting of an older male and an older female were included in analysis. Because male plumage does not change with age in this population of mountain bluebirds (Morrison et al. 2014), females were not responding to differences in colouration between first-year and older males.

Older females have more breeding experience than females in their first breeding season (Fowler 1995), so one possibility is that this experience enables them to gain

additional information signaled through a male's plumage. If true, an older, experienced female would be expected to bias the sex ratio of her offspring in response to her mate's attractiveness, while a younger, inexperienced female may not be able to respond to that information. Female age has been positively correlated with an increased proportion of sons in blue tits (Griffith et al. 2003), however, to my knowledge, no studies have yet found a relationship between female age, male attractiveness, and brood sex ratio.

Both male and female provisioning behaviour were related to brood sex ratio. During the early nestling phase, older males provisioned male-biased broods more frequently than female-biased broods. If provisioning behaviour reflects individual condition/quality, these results may suggest that female mountain bluebirds bias brood sex ratios in response to male condition. However, a female would not be aware of a male's provisioning behaviour prior to breeding, so she would have to be assessing other signals of male condition/quality. I found evidence to support the hypothesis that females adjust brood sex ratio in response to male attractiveness, so a potential explanation is that male plumage colouration is the signal linking male condition and provisioning behaviour. Male and female eastern bluebirds in better condition have brighter colouration and provision offspring at higher rates, suggesting that provisioning rates are condition dependent and reflected in plumage colouration (Siefferman and Hill 2003; Siefferman et al. 2005). However, there is no relationship between male colouration (i.e., condition) and provisioning rates in this population of mountain bluebirds (this study; also see Morrison et al. 2014).

Alternatively, provisioning rates may be related to territory quality. On high quality territories with easily accessible prey, males provision at increased rates (Keyser and Hill 2000). The quality of the territory a male is able to establish is dependent on his condition

and colouration (Keyser and Hill 2000). Female mountain bluebirds paired to male with brighter UV-blue rump plumage began laying earlier in the season, indicating that brighter males may be able to obtain high quality territories with enough resources to allow females to begin breeding earlier. In addition, older males are typically better able to secure high quality territories (Hill 1988). During the early nestling phase, older males and females provisioned male-biased broods at a higher rate than female-biased broods, suggesting that these pairs had nested in high quality habitat. However, no direct measures of habitat quality were available to further investigate this relationship. An important next step in deciphering this relationship would be to assess food availability and other indicators of habitat quality in each male's territory.

In a population of mountain bluebirds inhabiting the Bighorn Mountains, Wyoming, USA, pairs that nested at high elevation sites (2500 m) provisioned 28% more during the late nestling phase than pairs that nested at low elevation sites (1500 m) (Johnson et al. 2006). While the reason for this difference was unclear, it is possible that the parents were either compensating for the high thermoregulatory costs of living at high altitude, or that prey were more abundant or more easily accessible at higher elevations. If prey were more abundant, this may indicate that higher elevations provide better quality habitat. In my mountain bluebird population, parental provisioning rates did not change with elevation, though there was a significant relationship between elevation and sex ratio. The elevational gradient along which nest boxes were located was not nearly as great for my study (885-1116 m) as it was for Johnson et al. (2006), so the thermoregulatory costs of higher elevation nest sites are likely limited. Tree cover (range: 0–50%), however, did increase with elevation ($n = 38$, $\rho = 0.36$, $p = 0.03$), suggesting that habitat quality may increase with elevation within our study

area, as mountain bluebirds typically breed under moderate tree cover (Power and Lombardo 1996). If higher elevation sites provide higher quality habitat, this could help explain why I found more male-biased broods at higher elevations. Brood sex ratios have been shown to be modified in response to several indicators of habitat quality, including resource availability (Suorsa et al. 2003; Stauss et al. 2005), habitat structure (Suorsa et al. 2003), and intraspecific competition (Komdeur et al. 1997; Dickinson 2004).

Sex allocation theory predicts that females will adjust the sex ratio of their broods in response to the differential reproductive value of sons and daughters. Offspring reproductive value may vary as a result of mate attractiveness, mate condition, or territory quality; therefore, females should bias sex ratio in response to mate characteristics indicative of these attributes. As predicted based on sex allocation theory, I found that female mountain bluebirds produce more male-biased offspring sex ratios when paired with more attractive males (i.e., tail UV-blue plumage brightness). This relationship was dependent on female age, suggesting that a female's breeding experience may influence her ability to assess male plumage characteristics and respond accordingly. Brood sex ratio was also associated with elevation, suggesting the need for future studies to investigate the interactions between extrinsic and intrinsic factors in influencing offspring sex ratios. To my knowledge, this study is the only of its kind to find a relationship between female age, male attractiveness and brood sex ratio bias.

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APPENDIX A

Table A1. Results from a principal components analysis of measures of plumage colouration (brightness, hue and chroma). The first principal component (PC1) was used to represent overall variation in colour because it was found to explain most of the variation in plumage colouration for each plumage area

	Eigenvalue	Proportion of variance	Colour variable	Factor loading
Male tail PC1	1.88	0.63	Brightness	0.61
			UV + blue chroma	-0.60
			Hue	0.52
Female tail PC1	1.77	0.59	Brightness	0.54
			UV + blue chroma	-0.64
			Hue	0.55
Male rump PC1	2.2	0.73	Brightness	0.50
			UV + blue chroma	-0.59
			Hue	0.63
Female Rump PC1	2.09	0.7	Brightness	0.42
			UV + blue chroma	-0.64
			Hue	0.65

APPENDIX B

Table B1. Results of Spearman's rho correlations between brood sex ratio and male and female rump and tail (R3) plumage colouration. Data was analyzed both pooled and unpooled in regard to ASY and SY age classes for each sex separately.

	<i>n</i>	<i>ρ</i>	<i>p</i>
<i>Male plumage colouration</i>			
Pooled Ages			
R3 PC1	39	0.25	0.13
Rump PC1	39	0.11	0.50
ASY			
R3 PC1	29	0.23	0.23
Rump PC1	29	0.04	0.82
SY			
R3 PC1	10	0.46	0.18
Rump PC1	10	0.04	0.82
<i>Female plumage colouration</i>			
Pooled Ages			
R3 PC1	40	0.003	0.99
Rump PC1	40	-0.06	0.73
ASY			
R3 PC1	25	-0.11	0.60
Rump PC1	25	-0.11	0.60
SY			
R3 PC1	15	0.18	0.53
Rump PC1	15	0.005	0.98